# Stabilizing intramolecular cobalt–imidazole coordination with a remote methyl group in the backbone of a cofactor B12–protein model

Sonnay, Marjorie ; Zelder, Felix

Abstract: This communication describes the stabilizing effect ($\Delta\Delta G^\circ = -4$ kJ mol$^1$) of a remote methyl group in the backbone of a cobalamin–enzyme mimic on intramolecular imidazole–cobalt coordination. For this purpose, two B12 derivatives with an appended imidazole base were synthesized and analysed with spectrophotometric pH titrations. Qualitative conformation analysis of the backbone structure suggests that a thermodynamically unfavoured gauche interaction in the base-off form of a model containing an (R)-configured CH3 group at position C176 of the linker between the corrin ring and the terminal imidazole ligand steers the base toward cobalt coordination.

DOI: https://doi.org/10.1039/c8dt01298a

# Stabilizing Intramolecular Cobalt-Imidazole Coordination with a Remote Methyl Group in the Backbone of a Cofactor $B_{12}$-Protein Model

Dr. Marjorie Sonnay, and PD Dr. Felix Zelder*

*Department of Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. Fax: +41 44 635 6803; E-mail: felix.zelder@chem.uzh.ch, www.felix-zelder.net.*

**Abstract:** This communication describes the stabilizing effect ($\Delta\Delta G^0$ = - 4 kJ $mol^{-1}$) of a remote methyl group in the backbone of a cobalamin-enzyme mimic on intramolecular imidazole-cobalt coordination. For this purpose, two $B_{12}$ derivatives with an appended imidazole base were synthesized and analysed with spectrophotometric pH titrations. Qualitative conformation analysis of the backbone structure suggests that a thermodynamically unfavoured *gauche* interaction in the base-off form of a model containing an (*R*)-configured $CH_3$ group at position C176 of the linker between the corrin ring and the terminal imidazole ligand, steers the base toward cobalt coordination.

## Introduction:

The design and study of small molecular models of cofactor-protein active sites has contributed significantly to our current understanding of biological processes on a molecular level. As models of heme proteins, picket-fence porphyrins developed by the group of Collman represent probably the best studied and most famous example in this field.[1-3] These systems impressively described for the first time reversible dioxygen binding by introducing steric bulk in the periphery of the model compound. For models of vitamin $B_{12}$ ($B_{12}$; Figure 1), the study of `incomplete` corrinoids lacking the intramolecularly bound dimethylbenzimidazole base (Dmbz) at the *f*-side chain such as dicyano-, and

aquacyanocobinamides (Cbi) **1** and **2**$^{+}$ (Figure 2A) gave important insights into properties and reactivity of Co-containing corrin macrocycles.[4-10]

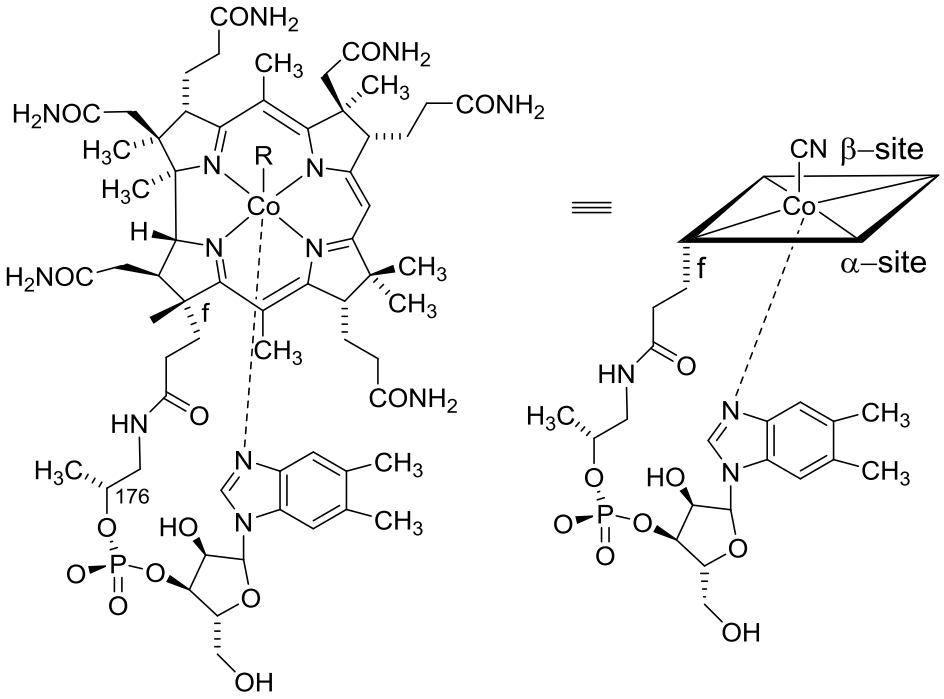



**Figure 1.** *Left*: Structures of Cbls (R = CN: cyanoCbl ($B_{12}$); R = $CH_3$: MeCbl; R = adenosyl: AdoCbl). *Right*: Schematic representation of $B_{12}$. The β-, and α-sites of the corrin macrocycle are indicated.

A variety of more sophisticated cobalamin (Cbl)-protein models have then been prepared by the groups of Rétey,[11, 12] Golding, [13, 14] Darbre and Keese[15-17], Hisaeda,[18, 19], Hayashi[20, 21] and others[22-27] to yield insights into $B_{12}$-catalyzed enzymatic reactions.

**Results and Discussion:**

My group is interested in studying the influence of α-axially coordinating ligands on the reactivity at the opposite β-site in Cbl-protein models.[28-30] These model studies are of interest since $B_{12}$ cofactors are attached to human $B_{12}$-dependent enzymes in two different ways: methylmalonyl CoA mutase (MCM) binds the AdoCbl cofactor (Figure 1 *left*) in the `base-on` form, while methionine synthase (MetH) binds MeCbl (Figure 1 *left*) in the so-called `base-off/histidine-on (His-on)` fashion (Scheme 1 *left*). In the latter case, the intramolecularly coordinated Dmbz base is replaced by an imidazole (Im) containing histidine residue of the protein. The impact of these two distinct binding modes for catalysis in Cbl-protein complexes has been controversially discussed[29, 31-33] Active sites of base-off/ His-on Cbl-

protein complexes have been first modelled with **3**$^{+}$ containing a coordinated Im ligand at the α-site of the Cbi complex (Figure 2B).[34] Our group introduced the intramolecular derivative **4**$^{+}$ as an optimized version of Cbl-protein models.[29] It consists of a corrin macrocycle, a peptide "loop" terminated with an imidazole (Im) base as α-coordinating ligand and a cyano group on the opposite axial β-site (Figure 2C).[29] In the model compound, the intramolecular Co-Im coordination mimics the cofactor`s attachment to the protein binding site, as schematically depicted in Scheme 1. The molecular structure of the backbone is decisive for tuning the strength of intramolecular coordination in the model compound. Despite this importance, little is known about how structural modifications of the backbone translate into differences in the intramolecular binding event. In this publication, we extend our earlier studies with biomimetic $B_{12}$-protein complexes[28, 29] and report on the influence of a remote conformational effect on intramolecular axial coordination of Im to the $Co^{III}$-centre. In particular, we demonstrate that the presence of a single methyl group located ten atoms from the cobalt center strengthens intramolecular coordination between the metal ion and the imidazole-anchoring group.

Inspired by earlier work of the groups of Eschenmoser, Kräutler and our own contributions on $B_{12}$ and its derivatives with a terminal Dmbz base,[35-37] we speculated whether a single (*R*)-configured methyl group located at position C176 of the imidazole-containing model **4**$^{+}$ would also lead to a stabilisation of the base-on form (**4**$^{+}$) over its base-off form (**4-H$_3$O$^{2+}$**) (Scheme 2).

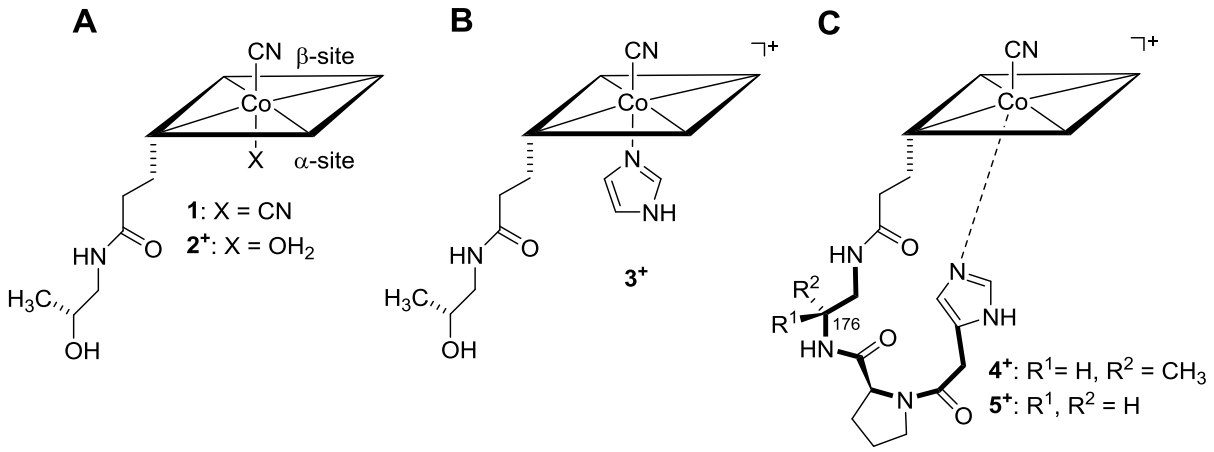
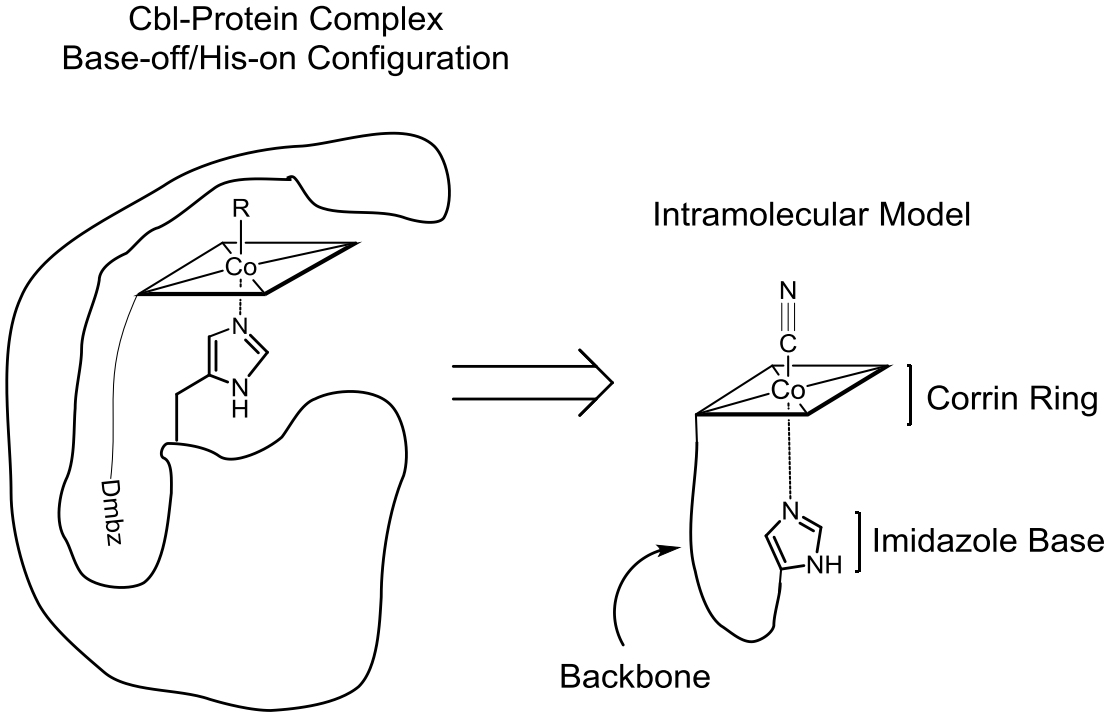


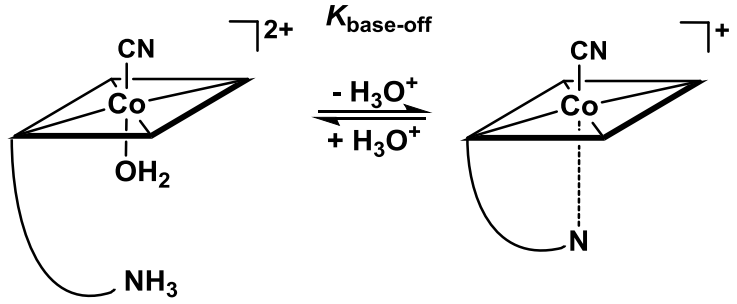

**Figure 2.** Structures of (A) Cbi **1**, **2**$^{+}$, (B) the intermolecular model **3**$^{+}$ and (C) the intramolecular models **4**$^{+}$, **5**$^{+}$.

For this purpose, we decided to synthesize the model compound **5**$^{+}$ (Figure 2C) lacking any substituent in the ethylene diamine subunit and compare its coordination properties to those of **4**$^{+}$ [29] with an (*R*)-configured methyl group at position C176.




**Scheme 1.** *Left:* Base-off/His-on bound Cbl in Cbl-protein complexes. *Right:* Schematic representation of an intramolecular model for Co-Im coordination in Cbl-protein complexes.

The biomimetic model **5**$^{+}$ was synthesized starting from cobyric acid and an Im-containing amino acid building block as outlined in the supporting information. The high-resolution mass spectrum of **5**$^{+}$ displayed a signal at m/z = 603.29267, a value consistent with $C_{58}H_{83}O_9N_{16}Co$ (calculated: m/z = 603.29250) which corresponds to $[M+H]^{2+}$. The UV-Vis spectrum of the compound with absorption maxima ($\lambda_{max}$ (log$\varepsilon$)) for the $\alpha$, $\beta$ and $\gamma$ bands at 555 (3.95), 523 (3.92) and 362 (4.49) nm is in excellent agreement with that of **4**$^{+}$ (556 (3.99), 522 (3.95) and 362 (4.49)[29]) suggesting that the intramolecularly bound imidazole bases of both compounds have similar geometries. The study of the compound coordination chemistry was achieved using spectrophotometric pH titration, which yielded to the determination of a p$K_{base\text{-}off}$ value of 2.4 ($R^2$ = 1.0) for compound **5**$^{+}$ (Scheme 2; Figure 3). The same measurement performed on **4**$^{+}$ resulted in a $pK_{base\text{-}off}$ of 1.7 ($R^2$ = 1.0)[29].




**4-$H_3O^{2+}$ or 5-$H_3O^{2+}$** **$4^+$ or $5^+$**

**Equation 1:** $K_{base-off} = (1+K_{Co})K_{Im}$

**Scheme 2.** *Top:* Base-on/base-off equilibria of the biomimetic models **$4^+$**, **4-$H_3O^{2+}$**, **$5^+$** and **5-$H_3O^{2+}$**. *Bottom:* Equation 1 for the calculation of the binding constant $K_{Co}$.[38]

These values are in agreement with previous studies of Dmbz-containing $B_{12}$-derivatives demonstrating that the presence of the (*R*)-configured C176 methyl group favours the base-on configuration through an impressive long distance constitutional effect.[36]

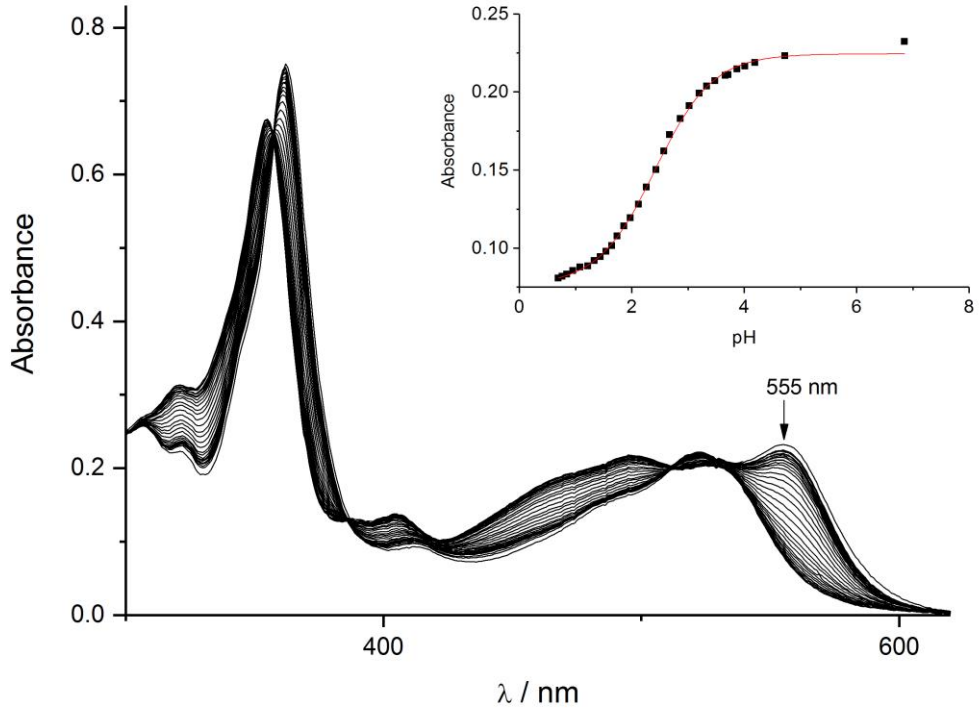



**Figure 3.** Spectrophotometric pH titration of **$5^+$** to **5-$H_3O^{2+}$** from pH 7.0 to pH 0.2. *Insert:* corresponding p$K_{base-off}$ determination plot (absorbance at 555 nm).

As depicted in Figure 4, the presence of a methyl group at C176 induces a supplementary *gauche* effect in the base-off configuration of **4-H$_3$O$^{2+}$**, while no additional unfavourable interactions are observed between base-on (**5$^+$**) and base-off (**5-H$_3$O$^{2+}$**) for the model **5$^+$** lacking any substituent in the ethylene diamine subunit. This difference thus explains that intramolecular coordination is thermodynamically favoured for **4$^+$** over **5$^+$** as indicated by its lower p$K_{base\text{-}off}$ value ($\Delta pK_{base\text{-}off}$ = 0.7; Table 1).

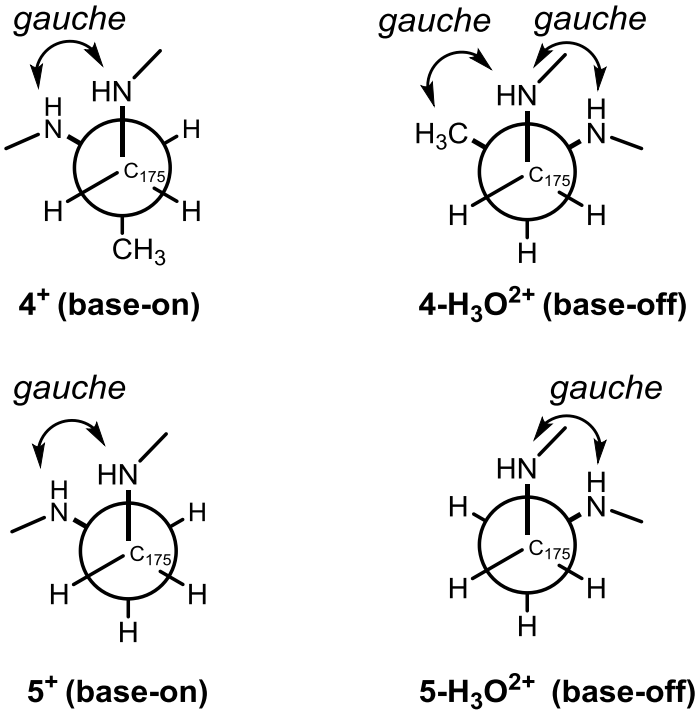



**Figure 4.** *Gauche* effects in the base-on configuration of **4$^+$** and **5$^+$** and the respective base-off configuration **4-H$_3$O$^{2+}$** and **5-H$_3$O$^{2+}$**.

The base-on/base-off equilibria of **4$^+$** and **5$^+$** are expressed in equation 1 (Scheme 2) and represent a composite of two consecutive equilibria for the deprotonation of the appended Im ligand, followed by coordination of the base to the metal center. The binding constant $K_{Co}$ for the latter process is calculated from the p$K_{base\text{-}off}$ values of the compounds and the p$K$a of the protonated Im base (p$K_a$ = 7.05[34])(Scheme 2).[39]

**Table 1.** Values of $K_{Co}$ and related thermodynamic constants for **4$^+$** and **5$^+$** (24 °C).

| Entry | Compound | p$K_{base\text{-}off}$ | $K_{Co}$ | $\Delta G^0$ |
|---|---|---|---|---|
| 2 | **4$^+$** | 1.7[a] | 2.2 x $10^5$ | -30 kJ mol$^{-1}$ |
| 3 | **5$^+$** | 2.4 | 4.5 x $10^4$ | -26 kJ mol$^{-1}$ |

[a]Ref. [29]

Compared to the strength of His-Co coordination in the intermolecular model $\mathbf{3}^{+}$ with a binding constant of 1.11 x $10^3$ $M^{-1}$,[34] the intramolecular model $\mathbf{5}^{+}$ exhibits a value of 4.5 x $10^4$ (Table 1). This binding constant translates to a free enthalpy ($\Delta G^0$) of -26 kJ $mol^{-1}$. Analysing the model $\mathbf{4}^{+}$ revealed a surprising strong effect of the remote methyl group on intramolecular coordination. The presence of a single (*R*)-configured methyl group at C176 shifts the equilibrium by a factor of ~ five towards metal-ligand coordination (base-on form; $K_{Co}$ = 2.2 x $10^5$; $\Delta G^0$ = -30 kJ $mol^{-1}$; Table 1). The difference in free enthalpy ($\Delta\Delta G^0$) of intramolecular Im-Co coordination between $\mathbf{4}^{+}$ and $\mathbf{5}^{+}$ of around 4 kJ $mol^{-1}$ is in excellent agreement with the observed rotational barrier between the *anti* and *gauche* conformations of propane due to a single *gauche* interaction ($\Delta\Delta G^0$ = 3.8 kJ $mol^{-1}$)[40]. This study demonstrates therefore strikingly that the understanding of subtle steric effects far away from the site of intramolecular metal-ligand coordination are important to understand biomimetic metal complexes on a molecular level.

## Conclusions:

In summary, biomimetic metal complexes of cofactor-protein active sites represent powerful structural and functional models of biological systems. This study underscores that remote steric effects play a role for defining the strength of intramolecular coordination in biomimetic metal complexes and hence, have to be considered. It is expected that this knowledge will be useful for the future design and optimisation of metal complexes mimicking metalloenzymes`active sites.

## Conflicts of interest

The authors declare no competing financial interest.


## Acknowledgement

The authors thank L. Bigler for the recording of the HR-ESI-MS spectra and R. Alberto for support. A generous gift of vitamin $B_{12}$ from DSM Nutritional Products AG

(Basel/Switzerland) is acknowledged. This work was supported by the Swiss National Science Foundation (Grant No. 200021 – 117822).

## Supporting Information

Supporting Information including the experimental details is available.

## Notes and References


1. J. P. Collman, R. R. Gagne, T. R. Halbert, J. C. Marchon and C. A. Reed, *J. Am. Chem. Soc.*, 1973, **95**, 7868-7870.
2. J. P. Collman and L. Fu, *Acc. Chem. Res.*, 1999, **32**, 455-463.
3. J. P. Collman, N. K. Devaraj, R. A. Decreau, Y. Yang, Y. L. Yan, W. Ebina, T. A. Eberspacher and C. E. D. Chidsey, *Science*, 2007, **315**, 1565-1568.
4. J. M. Pratt, ed., *Inorganic Chemistry of Vitamin B12*, Academic Press, New York, 1972.
5. H. M. Marques, J. C. Bradley, K. L. Brown and H. Brooks, *J. Chem. Soc. Dalton Trans.*, 1993, 3475-3478.
6. K. S. Conrad and T. C. Brunold, *Inorg. Chem.*, 2011, **50**, 8755-8766.
7. C. Männel-Croisé and F. Zelder, *Inorg. Chem.*, 2009, **48**, 1272-1274.
8. B. Aebli, C. Männel-Croisé and F. Zelder, *Inorg. Chem.*, 2014, **53**, 2516-2520.
9. K. Zhou and F. Zelder, *J. Porphyrins Phthalocyanines*, 2011, **15**, 555-559.
10. K. Zhou and F. Zelder, *Eur. J. Inorg. Chem.*, 2011, 53-57.
11. E. Retey, eds. B. Kräutler, D. Arigoni and B. Golding, Wiley-VCH, Weinheim, 1998, p. 274.
12. L. Poppe, H. Bothe, G. Broker, W. Buckel, E. Stupperich and J. Retey, *J. Mol. Cat. B*, 2000, **10**, 345-350.
13. B. T. Golding, C. S. Sell and P. J. Sellars, *J. Chem. Soc. Perk. Trans. 2*, 1980, 961-970.
14. P. Friedrich, U. Baisch, R. W. Harrington, F. Lyatuu, K. Zhou, F. Zelder, W. McFarlane, W. Buckel and B. T. Golding, *Chem. Eur. J.*, 2012, **18**, 16114-16122.
15. R. Keese, T. Darbre, U. v. Arx, S. Müller, A. Wolleb-Gygi, D. Hirschi, V. Siljegovic, M. Pfammatter, A. Amolins and T. Otten, eds. B. Kräutler, D. Arigoni and B. Golding, Wiley-VCH, Weinheim, 1998, p. 290.
16. M. J. Pfammatter, T. Darbre and R. Keese, *Helv. Chim. Acta*, 1998, **81**, 1105-1116.
17. C. Wedemeyer-Exl, T. Darbre and R. Keese, *Org. Biomol. Chem.*, 2007, **5**, 2119-2128.
18. L. Pan, K. Tahara, T. Masuko and Y. Hisaeda, *Inorg. Chim. Acta*, 2011, **368**, 194-199.
19. L. Pan, H. Shimakoshi, T. Masuko and Y. Hisaeda, *Dalton Trans.*, 2009, 9898-9905.
20. T. Hayashi, Y. Morita, E. Mizohata, K. Oohora, J. Ohbayashi, T. Inoue and Y. Hisaeda, *Chem. Commun.*, 2014, **50**, 12560-12563.
21. Y. Morita, K. Oohora, E. Mizohata, A. Sawada, T. Kamachi, K. Yoshizawa, T. Inoue and T. Hayashi, *Inorg. Chem.*, 2016, **55**, 1287-1295.
22. L. Hannibal, S. D. Bunge, R. van Eldik, D. W. Jacobsen, C. Kratky, K. Gruber and N. E. Brasch, *Inorg. Chem.*, 2007, **46**, 3613-3618.
23. M. S. A. Hamza, X. Zou, R. Banka, K. L. Brown and R. van Eldik, *Dalton Trans.*, 2005, 782-787.
24. M. Fasching, W. Schmidt, B. Kräutler, E. Stupperich, A. Schmidt and C. Kratky, *Helv. Chim. Acta*, 2000, **83**, 2295-2316.
25. B. E. Daikh and R. G. Finke, *J. Am. Chem. Soc.*, 1992, **114**, 2938-2943.
26. B. Kräutler, M. Hughes and C. Caderas, *Helv. Chim. Acta*, 1986, **69**, 1571-1575.
27. S. M. Chemaly, M. Florczak, H. Dirr and H. M. Marques, *Inorg. Chem.*, 2011, **50**, 8719-8727.
28. C. Männel-Croisé and F. Zelder, *Chem. Commun.*, 2011, **47**, 11249-11251.
29. M. Sonnay, T. Fox, O. Blacque and F. Zelder, *Chem. Sci.*, 2016, **7**, 3836-3842.

30. F. Zelder, *J. Porphyrins Phthalocyanines*, 2018, DOI: 10.1142/S1088424611003446, accepted
31. C. L. Drennan, S. Huang, J. T. Drummond, R. G. Matthews and M. L. Ludwig, *Science*, 1994, **266**, 1669-1674.
32. T. A. Stich, A. J. Brooks, N. R. Buan and T. C. Brunold, *J. Am. Chem. Soc.*, 2003, **125**, 5897-5914.
33. K. S. Conrad, C. D. Jordan, K. L. Brown and T. C. Brunold, *Inorg.Chem.*, 2015, **54**, 3736-3747.
34. G. I. H. Hanania, D. H. Irvine and M. V. Irvine, *J Chem Soc A*, 1966, 296-299.
35. A. Eschenmoser, *Angew. Chem. Int. Edit.*, 1988, **27**, 5-39.
36. P. Butler, M. O. Ebert, A. Lyskowski, K. Gruber, C. Kratky and B. Kräutler, *Angew. Chem. Int. Ed.*, 2006, **45**, 989-993.
37. K. Zhou and F. Zelder, *Angew. Chem. Int. Ed.*, 2010, **49**, 5178-5180.
38. K. L. Brown, *Chem. Rev.*, 2005, **105**, 2075-2149.
39. K. L. Brown, J. M. Hakimi and D. W. Jacobsen, *J. Am. Chem. Soc.*, 1984, **106**, 7894-7899.
40. A. Streitwieser and C. H. Heathcock, Verlag Chemie, Weinheim, 1980.